FDDU
Procedures for Receiving Collection Kits and Sample Plate Preparation

1 Scope

These procedures apply to DNA personnel who perform sample receipt or check in, submission management, plate creation, plate preparation, and/or punch collection kits received by the Federal DNA Database Unit (FDDU). The FDDU may receive three types of collection kits: a buccal collection kit; a liquid blood collection kit, which contains a liquid blood tube; or a finger stick collection kit, which contains dried bloodstain card(s).

2 Equipment/ Materials/ Reagents

Equipment/Materials

- General Laboratory Supplies
- STACS (Sample Tracking and Control System) Software (STACS DNA Inc.) version 6.0 or above
- Barcode printer with appropriately sized labels (2.0" x 0.5" or equivalent)
- Barcode Scanner (Honeywell or equivalent)
- 96-Well Sample (MicroAmp) Plates (aka PCR Plate) (Applied BioSystems or equivalent)
- Plate Sealer, microplate (Agilent PlateLoc or equivalent) with heat seal
- Optical Adhesive Covers (Applied BioSystems or equivalent)
- RFID Tags, Reader and software (VISI-TRAC RFID or equivalent)
- Bloodstain card (Whatman® FTA® Genecard or equivalent)
- Sample storage pouches (Fitzco or equivalent)
- Agilent Robotic Workstation (Agilent Bravo)
- Agilent Vworks Software, version 11.2 or higher
- Punch Instrument (BSD600-Duet, BSD 600Plus, or equivalent)

Reagents

- SwabSolution™ Kit (Promega)
- Prep-N-Go Buffer (Applied Biosystems)
- Water, Reagent Grade (VWR or equivalent)
- Buffer, Low TE (aka TEKnova DNA Suspension Buffer) (Fisher Scientific or equivalent)

3 Standards and Controls

Two Combo controls (aka Negative) and two Blood/Buccal Internal Standard (BIS) controls are included on each amplification plate.

The BIS is prepared as described in the DNA QA procedure for reagents (i.e., DNAQA 609) and is added to the plate during the sample punch procedure. These controls will be interpreted according to the criteria in the applicable FDDU Procedure (i.e., FDDU 315).

4 Procedures

4.1 Receipt of Sample Collection Kits

FDDU collection kits are shipped to the FBI Laboratory via US Postal Service Business Reply Mail and, on occasion, by an express mail service. The kits are delivered to the FDDU by FBI Laboratory mailroom personnel or picked up from the mailroom by FDDU personnel.

4.1.1 Using STACS, record kits received by the FDDU by scanning the FDDU collection kit barcode affixed to the collection kit. If necessary, barcodes may be hand-entered.

4.1.1.1 If kits are not logged into STACS on the same day as received, the date received should be recorded on a collection kit, batch, or bin, as appropriate, until they are logged into STACS.

4.1.1.2 When all received kits have been scanned each batch or bin of kits, as appropriate, should be labeled with the date received.

4.1.2 Proceed to **Check In and Barcoding** or place the FDDU collection kits in storage. Store kits containing buccal or dried bloodstain samples at room temperature and kits containing blood tubes refrigerated.

Place kits containing barcoded samples into storage at room temperature. Place each storage container containing barcoded blood tubes into refrigerated storage. Store the FD-936 forms in a designated area.

4.2 Check In and Barcoding of Collection Kits

Retrieve kits to be checked in. Kits should be processed in order of the date received. Each person performing the check in procedures must only have one kit open at a time and process the kits individually.

4.2.1 Scan the FDDU collection kit barcode. Inspect the kit for integrity issues (e.g. damaged packaging or lacking a tamper-evident seal). Integrity issues may result in a kit being marked as unacceptable.

4.2.2 Open the kit. Each kit must contain the following:

a. Completed FD-936 form.

b. FDDU Sample(s) (e.g., buccal collection device, blood sample in an EDTA

vacutainer tube, dried bloodstain card(s))

4.2.3 Ensure that the same subject name or alternate unique identification number (e.g., FBI#, SSN, Alien#, BOP#, FINS#, collection device/card barcode(s)) is on the FD-936 and the corresponding sample.

4.2.4 STACS may auto-populate the following fields; however, the user may manually enter or change the entries, if necessary.

- Contributor Type
- Submitting Agency
- Specimen Nature
- Count

4.2.5 Determine if the kit is deemed acceptable. If not, select a reason from the pull-down menu. If an applicable reason is not available in the pull-down menu, "Other" may be selected and the reason entered in the appropriate field. Check in and barcoding is completed for all kits; however, unacceptable kits will be evaluated according to applicable FDDU Procedure (i.e. FDDU 302).

4.2.6 Additional information may be entered into the comment field, if necessary.

4.2.7 Upon completion of the sample check in, STACS prints a set of barcodes. Place the appropriate barcode labels on the FD-936 and each sample. Ensure that the barcode label placed on the FD-936 corresponds to the barcode label placed on the respective sample(s).

4.2.8 Place a RFID tag on each sample.

4.2.9 Scan the STACS barcodes and the RFID tags to associate them in STACS.

***NOTE**: If necessary, the RFID software may be used to associate the sample(s) to the RFID tag(s).*

4.2.10 Place the sample(s) (e.g., buccal cards, blood cards, buccal cassettes) into a sample storage pouch for storage. Place blood tubes in the designated storage location.

4.2.11 Collection devices (if provided) may be properly discarded following check in of the collection kit.

Repeat above steps to check in each additional kit.

4.2.12 Place all packaged samples into an appropriate storage container (e.g., box or plastic sleeve) labeled with an RFID container tag.

4.2.13 Verify the contents of each storage container using an RFID reader. If necessary, resolve any discrepancies regarding the inventory of the container.

4.2.14 Place each storage container containing barcoded samples into storage at room temperature. Place each storage container containing barcoded blood tubes into refrigerated storage. Store the FD-936 forms in a designated area.

4.3 Blood Spotting

The FDDU occasionally receives liquid blood samples, which must be manually dried onto a bloodstain card (e.g., FTA Genecard) prior to initiating the DNA analysis procedure.

4.3.1 Prior to spotting a liquid blood sample, the corresponding FTA card must be labeled with the appropriate STACS barcode and RFID tag.

4.3.2 Retrieve each blood tube from refrigerated storage and allow to come to room temperature. Invert the blood tube several times before processing.

4.3.3 Scan the matching STACS barcodes affixed to the blood tube and FTA card.

4.3.4 Spot approximately 50 µl of the liquid blood onto each circle on the FTA card in a laminar flow hood.

4.3.5 Discard the liquid blood tube in the appropriate biohazard container.

4.3.6 Allow the bloodstain card to dry for approximately 1 hour.

4.3.7 Place the bloodstain card and a desiccant pouch into a sample storage pouch.

4.3.8 Place all packaged bloodstain cards into an appropriate storage container labeled with an RFID container tag.

4.3.9 Place each storage container containing barcoded bloodstain cards into storage at room temperature or in a freezer.

4.4 Plate Creation

All accepted samples are available for processing based on the selection of the amplification kits. Blood samples may be analyzed using the GlobalFiler Express amplification kit. Buccal samples may be analyzed with the Identifiler Direct or GlobalFiler Express amplification kits. Once an amplification kit is selected, the sample(s) are available to be selected for a plate and processed in the laboratory.

4.4.1 Select and gather the appropriate samples to be allocated to the plate.

4.4.2 STACS may prompt the user to populate the following fields as appropriate:

a. Plate Cycle Number – 25, 26, 27, or 28
b. Plate Punch Size – 1.2 mm

4.4.3 Upon completion of plate creation, STACS prints out unique plate barcodes. Place each barcode accordingly, on the PCR plate and support base.

4.4.4 Scan the barcodes affixed to both the PCR plate and support base. STACS verifies the scanned barcodes.

4.5 Plate Preparation

Refer to DNA Procedure Introduction (DNA QA 600) for applicable laboratory quality assurance and cleaning instructions.

4.5.1 Select appropriate plates to be processed and indicate whether the plate preparation process will be done manually or using the Agilent Bravo. If using the Agilent Bravo, load the plates onto the instrument deck.

4.5.2 Scan the barcodes affixed to each plate and the reagent(s) required for the selected scenario. If using the Agilent Bravo, scan the instrument, click process in STACS and follow prompts from associated VWorks software.

4.5.3 Add appropriate anti-static reagent to the plate(s), if required.

a. Identifiler Direct - Add 2µl of reagent grade water. This combination may be performed manually or using the Agilent Bravo Robotic Workstation.

b. GlobalFiler Express (FTA Paper) - Add 3µl of DNA Suspension Buffer (Low TE Buffer). This combination may be performed manually or using the Agilent Bravo Robotic Workstation.

c. GlobalFiler Express (Non-FTA Paper) - Add 3µl of Prep-N-Go Buffer. This combination may be performed manually or using the Agilent Bravo Robotic Workstation.

d. GlobalFiler Express (FTA Paper and Non-FTA Paper) – Add 3µl of SwabSolution. This combination may be performed manually or using the Agilent Bravo Robotic Workstation.

NOTE: *Alternatively, for Identifiler Direct Plate Prep, 4µl of reagent grade water, 2µl of Prep-N-Go buffer, or no anti-static reagent are also suitable. These may be added manually or using the Agilent Bravo Robotic Workstation.*

4.5.4 Select whether the plate preparation process was successful, failed, or aborted. Comments and observations should be entered for plates with process failed or aborted results.

4.6 Sample Punch

The following summarizes the combinations validated for sample processing:

Sample Type	Punch Size	Amplification Kit	Amplification Cycles
Blood	1.2mm	GlobalFiler Express	26 or 28
Buccal	1.2mm	Identifiler Direct	25, 26, 27 or 28
		GlobalFiler Express	26 or 28

4.6.1 Ensure necessary cleaning procedures are performed on the punch instrument prior to use.

4.6.2 Select a created plate.

4.6.2.1 Scan and punch the samples or controls into each of the allocated wells on the punch instrument in the order displayed in STACS.

NOTE: When punching samples on a BSD, ensure that the required "Cleaning Strike(s)" will be placed in between each sample by using the appropriate designation in the BSD Configuration file. Punch the "cleaning sample(s)" (e.g., clean FTA card, clean filter paper) when prompted.

4.6.3 Upon completion of punching a plate:

- Visually inspect the plate to verify its integrity.
- Indicate the result as successful, failed, or aborted.
- Comments and observations must be entered for plates with process failed results.

4.6.4 Cover successfully punched plates and transfer them to the laboratory for processing.

5 Sampling

A reasonable assumption of homogeneity can be made for database samples; therefore, any sampling (i.e., punch) will be considered representative of the entire sample.

6 Calculations

Not applicable.

7 Measurement Uncertainty

Not applicable.

8 Limitations

Only the following combinations are approved for sample processing:

Sample Type	Plate Prep	Punch Size	Amplification Kit	Amplification Cycles
Blood (FTA)	3ul of DNA Suspension Buffer (Low TE)	1.2mm	GlobalFiler Express	26 or 28
Buccal (FTA)	Nothing or 2µl or 4µl water or 2µl Prep-N-Go	1.2mm	Identifiler Direct	25, 26, 27 or 28
	3µl of DNA Suspension Buffer (Low TE)	1.2mm	GlobalFiler Express	26 or 28
	3µl of SwabSolution	1.2mm	GlobalFiler Express	26
Buccal (Non-FTA)	3µl of Prep-N-Go Buffer	1.2mm	GlobalFiler Express	26 or 28
	3µl of SwabSolution	1.2mm	GlobalFiler Express	26

9 Safety

9.1 All FDDU samples that contain blood are considered potentially infectious regardless of the perceived status of the source individual or the age of the material. All FDDU personnel who work with such material will follow the "Bloodborne Pathogen Exposure Control Plan" found in the most current version of the *FBI Laboratory Safety Manual*.

9.2 Refer to the “Safe Work Practices and Procedures”, “Bloodborne Pathogen Exposure Control Plan”, “Personal Protective Equipment”, and “Chemical Hygiene Plan” sections of the *FBI Laboratory Safety Manual* for important personal safety information.

9.3 Refer to the “Hazardous Waste Disposal” section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures as well as the biohazardous wastes generated.

10 References

FBI Laboratory Quality Assurance Manual.

FBI Laboratory Operations Manual.

FBI Laboratory Safety Manual.

DNA Procedures Manual.

STACS DNA Inc. *Sample Tracking and Control System (STACS) User's Guide*.

Symbol Technologies Inc. *Symbol Barcode Reader User's Guide*.

Zebra Technologies International. *Zebra Barcode Printer User's Guide*.

BSD600-Duet Semi-Automated Dried Sample Punch Instrument Operator Guidelines (BSD Robotics)

Rev. #	Issue Date	History
5	03/11/16	Revised entirety of document for simplification and clarity and to remove software interface instructions. Added procedures for plate punch previously in 304-7.
6	12/09/16	Revised to incorporate GlobalFiler Express and non-FTA buccal samples. Changed cards and sample cards to samples throughout.
7	02/28/18	Changed STACS version to 6.0 Removed instructions for the Wallac punch instrument and Identifiler amplification kit throughout. Changed 4°C to refrigerated and -20°C to frozen throughout. 4.3: Added blood spotting instructions, renumbered remaining sections.
8	09/13/19	Added SwabSolution procedures throughout. 1 Update scope 2 Added BSD 600Plus 3 Added info to Standards and Controls 4.1 Added how samples are transported to and receive by the lab. 4.2.5 Added additional info for unaccepted kits. 4.4.1 Relocated gathering samples. 4.4.2 Removed 24 cycle option missed on Rev 7 and punch count. 5 Added Sampling info. 8 Updated table.

Approval

Redacted - Signatures on File

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QA Approval

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